Temperature and Seed Moisture Govern Storage Duration and Germination of *Phlox drummondii*

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Abstract. *Phlox drummondii* Hook seed germinated well over a wide range of constant or alternating temperatures. Germination percentages at constant temperatures of 10 to 25C ranged from 94% to 98% for 'Light Salmon' and from 83% to 88% for 'Deep Salmon'. No seeds germinated at constant 30C. Total germination percentages at alternating temperatures ranged from 93% to 99% for 'Light Salmon' and 67% to 82% for 'Deep Salmon'. For both cultivars, the number of days to 50% of final germination (T_{50}) and between 10% and 90% germination ($T_{90} - T_{10}$) decreased as constant temperatures or the median for alternating temperatures rose from 10 to 20C. The seeds had only limited desiccation tolerance. Reducing the moisture content of stored seed from 9% to 5% did not reduce total germination significantly, but 5% to 6% seed moisture levels increased the days to T_{50} and $T_{90} - T_{10}$ compared with higher moisture contents. The relative humidity and temperature that phlox seed received during long-term storage influenced germination. After seed was stored at 5C, germination generally was higher, earlier, and more uniform than after storage at 15 or 25C. The highest total germination percentages and shortest T_{50} and $T_{90} - T_{10}$ occurred following storage for 12 months at 5C and 20% to 40% relative humidity.

Annual phlox are colorful landscape plants, and recently introduced dwarf strains are gaining popularity as flowering bedding plants. Temperature is critical for maximum germination; Ball (1985) recommends 16C and Shirokova et al. (1977) recommend 20C. Cathey (1976) reported that continuous darkness was required to achieve maximum total germination. Jana and Dass (1984) found that pretreating phlox seed by soaking them in growth regulator solutions increased subsequent germination in darkness. Heydecker and Hendy (1975) reported that priming *Phlox drummondii* seed in 33.4% polyethylene glycol 8000 at 15.5C for 14 days promoted faster and more uniform germination. Research on storing annual phlox seed has not been conducted. Our research objectives were to compare germination at constant and alternating temperatures, determine the effects of desiccation on seed viability, and measure temperature and relative humidity (RH) interactions during seed storage.

Seed handling and germination procedures. Seeds of 'Light Salmon' and 'Deep Salmon' *Phlox* Palona were received from Sluis and

Groot Quality Seeds, Enkhuizen, Holland, 6 Nov. 1990, 4 weeks after harvest. Seeds were dusted with 3a, 4, 7, 7a-tetrahydro-2-[(trichloromethyl)thio]-1H-isoindole-1,3(2H)-dione (captan). Experimental units consisted of four 100-seed replicates, which were germinated in individual 9-cm petri dishes on a double layer of Whatman no. 1 filter paper moistened with 6 ml of distilled water. Seeds were germinated in incubators (Stults Scientific Engineering Corp, Springfield, Ill.) at constant or 12-h alternating temperatures. In each study, germination percentage (G), days to 50% of final germination (T_{50}), and germination span in number of days between 10% and 90% germination ($T_{90} - T_{10}$) were calculated as described by Furutani et al. (1985).

Germination temperature. 'Light Salmon' and 'Deep Salmon' seeds were germinated in petri dishes placed in dark incubators at constant 10, 15, 20, 25, or 30C. In a second study, seeds were stored at 5C for 3 weeks before they were germinated in darkness at 12-h alternating temperatures of 10/15C, 10/20C, 15/20C, 15/25C, or 20/25C. Seeds with radicles emerged through the testa were counted daily. Trends across the temperatures were determined for G, T_{50} , or $T_{90} - T_{10}$ by fitting the data using polynomial regression equations. The criterion used for determining the best-fitting regression equation was to perform a significance test on the coefficient estimate of the highest-degree term in the model. A curvilinear trend was detected by finding a significant ($P < 0.05$) quadratic coefficient estimate. A trend that was not curvilinear but strictly linear was detected by fitting a simpler model form

and finding a significant ($P < 0.05$) linear or slope coefficient estimate.

Seed moisture content. The seed replications of both cultivars were weighed, placed in 9-cm open petri dishes, and dehydrated for 1, 6, 12, 24, 48, or 72 h in 40C forced-draft ovens. After being dehydrated, each replication was reweighed and immediately sealed in screw-capped 10-ml glass vials, 100 seeds per vial, and stored at 5C for 3 weeks. After storage, seeds were reweighed and germinated in darkness at constant 20C as previously described. Seeds with emerged radicles were counted daily, and germination data were analyzed by fitting the data using polynomial regression equations. When the seeds were received, four 100-seed lots of each cultivar were weighed, dehydrated at 105C for 48 h, and reweighed after they had cooled to determine the initial moisture content.

Temperature and RH interactions during seed storage. 'Light Salmon' seeds were stored in 15- × 2.5-cm petri dishes on wire screens supported by segments of tubing 1 cm above a chemical-desiccant. Humidity was controlled using 50-ml of saturated lithium chloride, magnesium chloride, magnesium nitrate, sodium chloride, or potassium nitrate to achieve 11%, 34%, 52%, 75%, or 95% RH, respectively (Copeland, 1976). Incubators with refrigeration and heating capacities maintained a constant 5, 15, or 25C during 3, 6, 9, or 12 months of seed storage. After storage, seeds from all treatments were germinated in darkness at 20C as described previously. Also, groups of four replications of 100 seeds each were desiccated for 48 h in forced-draft ovens at 105C to determine seed moisture content after storage at each RI-I. The design was a 5 × 3 × 4 factorial with four replicates of each treatment. The data were tested by analysis of variance and fitting multiple regression equations using SAS's PROC REG program (SAS, 1985) to determine the effects of temperature, storage period (SP), and RH on germination. Initially, the model that was fitted at each level of storage temperature was a complete second-degree polynomial: Response = $\beta_0 + \beta_1 SP + \beta_2 RH + \beta_3 SP \times RH + \beta_4 SP^2 + \beta_5 RH^2 + E$, where $\beta_1 SP$ and $\beta_2 RH$ represent the linear effects of SP and RH, respectively, on the response; $\beta_3 SP \times RH$ represents a linear-by-linear interaction effect between SP and RH; and $\beta_4 SP^2$ and $\beta_5 RH^2$ represent the curvilinear (quadratic) effects of SP and RH. Higher-degree terms that did not attain a significance level of $P = 0.05$, such as $\beta_4 SP^2$ and $\beta_5 RH^2$, were dropped to produce simpler, reduced model forms in some cases. Plots of the estimated germination surfaces were generated using the CONTOUR function in the S language (Becker et al., 1988).

The averages of the measured responses (G, T_{50} , and $T_{90} - T_{10}$) at constant and alternating temperatures for 'Light Salmon' and 'Deep Salmon' are listed in Tables 1 and 2, respectively. Also listed are the coefficient estimates for the fitted regression equation, response = $b_0 + b_1 \text{temp} + b_2 \text{temp}^2$, if a curvilinear trend was detected, or, if only a linear trend was present, response = $b_0 + b_1 \text{temp}$. Results of the

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significance tests performed on the estimates are also reported in the tables. For alternating temperatures, the median temperature was used in fitting the regression equation.

Total G remained relatively constant for 'Light Salmon' (94% to 98%) and 'Deep Salmon' (83% to 88%) from 10 to 25C (Table 1). No seeds germinated at 30C (data not presented). For both cultivars, the number of days to T_{50} decreased significantly ($P < 0.001$) as germination temperatures increased from 10 to 20C. For both cultivars, temperature had a curvilinear effect: T_{50} increased slightly at 25C over that at 20C. The span $T_{90} - T_{10}$ decreased for 'Light Salmon' as temperatures increased from 10 to 20C, and then increased at 25C. For 'Deep Salmon', the span decreased linearly ($P < 0.01$) over the entire range of

temperatures. Total G at alternating temperatures ranged from 93% to 99% for 'Light Salmon' and 67% to 82% for 'Deep Salmon' (Table 2). 'Light Salmon' total G showed no trend at the alternating temperatures studied. The number of days to T_{50} and the germination span ($T_{90} - T_{10}$) decreased linearly for both cultivars as the median alternating temperature increased (Table 2).

The moisture content of 'Light Salmon' seed during storage affected germination in darkness at 20C (Table 3). While a slight ($P = 0.07$) linear decrease in total G was observed for decreasing seed moisture content from 9% to 5%, days to T_{50} and $T_{90} - T_{10}$ increased curvilinearly. The values of T_{50} and $T_{90} - T_{10}$ remained constant at 9% to 7% but increased at 6% and 5% moisture levels.

There were no significant changes in G , T_{50} , or $T_{90} - T_{10}$ after storing 'Light Salmon' or 'Deep Salmon' seeds at 5, 0, -5, -10, -15, or -20C for 4 weeks (data not shown). 'Light Salmon' G varied among these storage temperatures from 99% to 97%, T_{50} from 3.3 to 3.6 days, and $T_{90} - T_{10}$ from 2.6 to 3.0 days. These results indicate that phlox seed are very cold-tolerant, and long-term storage at subzero temperatures may be possible.

The effects of SP and RH on G, T_{50} , and $T_{90} - T_{10}$ at each storage temperature (5, 15, or 25C), are reflected in the plots of surface contours (Fig. 1) and the coefficient estimates of the fitted regression models (Table 4). Variation in the observed total G, when taken across the four petri dishes, remained constant over the wide range (10% to 100%) of G values

Table 1. Germination of phlox seed under constant temperatures in darkness.

Cultivar	Temp (°C)	Germination ^a			Regression coefficient estimates								
		G	T_{50}	$T_{90} - T_{10}$	G			T_{50}			$T_{90} - T_{10}$		
		(%)	(days)	(days)	b_0	b_1		b_0	b_1	b_2	b_0	b_1	b_2
Light Salmon	10	97	8.6	5.3	95.1	0.08		22.6	-1.83	0.04	12.2	-0.88	0.02
	15	94	4.5	3.6									
	20	98	3.3	2.6									
	25	97	3.4	2.8									
	Linear	NS	***	***									
Deep Salmon	10	83	9.2	6.0	80.9	0.28		18.4	-1.20	0.03	6.4	-0.05	
	15	86	6.7	5.5									
	20	88	5.8	5.2									
	25	87	6.0	5.1									
	Linear	NS	***	*									
	Quadratic	NS	**	NS									

^aData are means of 400 seeds.

NS,*,**,*Nonsignificant or significant at $P = 0.05, 0.01, \text{ or } 0.001$, respectively.

Table 2. Germination of phlox seed under alternating temperatures in darkness.

Cultivar	Temp (°C)		Germination			Regression coefficient estimates					
	Alternating	Median	G	T_{50}	$T_{90} - T_{10}$	G		T_{50}		$T_{90} - T_{10}$	
			(%)	(days)	(days)	b_0	b_1	b_0	b_1	b_0	b_1
Light Salmon	10-15	12.5	98	5.3	3.1	99.2	-0.12	6.4	-0.11	4.4	-0.10
	10-20	15.0	99	4.8	3.2						
	15-20	17.5	96	4.1	2.5						
	15-25	20.0	93	4.5	2.5						
	20-25	22.5	99	4.1	2.2						
Deep Salmon	10-15	12.5	82	7.1	5.3	97.5	-1.18	8.7	-0.12	7.1	-0.13
	10-20	15.0	78	7.0	5.3						
	15-20	17.5	80	6.2	4.8						
	15-25	20.0	78	6.8	4.6						
	20-25	22.5	67	5.7	4.1						
Linear			*	**	***						
	Quadratic		NS	NS	NS						

^aData are the means of 400 seeds.

NS,*,**,*Nonsignificant or significant at $P = 0.05, 0.01, \text{ or } 0.001$, respectively.

Table 3. Effect of seed moisture content on the germination of 'Light Salmon' phlox seed at 20C in darkness.

Seed moisture (%)	Germination			Regression coefficient estimates								
	G	T_{50}	$T_{90} - T_{10}$	G			T_{50}			$T_{90} - T_{10}$		
	(%)	(days)	(days)	b_0	b_1		b_0	b_1	b_2	b_0	b_1	b_2
9	98	5.7	2.5	80.6	2.0		10.4	-1.17	0.07	6.8	-1.03	0.06
8	98	5.6	2.5									
7	95	5.7	2.6									
6	94	5.9	2.9									
5	89	6.3	3.2									
Linear	NS	***	**									
Quadratic	NS	**	*									

NS,*,**,*Nonsignificant or significant at $P = 0.05, 0.01, \text{ or } 0.001$, respectively.

observed. Estimated G values of $\geq 95\%$ occurred after storage at 5C and 20% to 40% RH, while estimated G values $>90\%$ occurred after 6 months of storage at $<75\%$ RH and up to 12 months of storage at $<60\%$ RH (Fig. 1A). Estimated G values declined when RH was $>40\%$ for >8 months. At 15C, germination was 100% after seed storage at 35% to 55% RH for ≤ 4 months, but estimated G values decreased more rapidly at higher RHs ($\geq 60\%$) and longer storage periods (≥ 8 months) at 15 than at 5C (Fig. 1B). Seeds stored at 25C had the lowest total G after storage among the three temperatures studied (Fig. 1C). At 25C, the highest estimated total G was only slightly $>80\%$ at RHs $\leq 60\%$ and SPs <6 months, or when seeds

were stored >6 months at an RH $\leq 35\%$.

When seed was stored at 5C, the estimated T_{50} was <4 at an RH $\leq 60\%$ and SPs <6 months (Fig. 1D). The T_{50} remained <4 days for all SPs when RHs were $\leq 40\%$. Seeds stored at 15C had slightly higher T_{50} values than at 5C for similar RHs and SPs (Fig. 1E). The T_{50} remained low (≤ 4 days) over 12 months of storage at $<20\%$ RH, or at 0% to 60% RH for an SP of <5 months. At 25C, T_{50} was lowest (14 days) only after storage for <5 months at an RH $<40\%$ (Fig. 1F). The estimated T_{50} following storage at 25C increased dramatically as SPs and RHs increased to >8 months and $>60\%$, respectively.

The $T_{90}-T_{10}$ span increased with high SPs

and RHs, at which similar patterns developed at the three storage temperatures (Fig. 1G-I). The lowest $T_{90}-T_{10}$ (12 days) occurred after <4 months storage at 5C or after <3 months at 15C at 10% to 60% RH (Fig. 1G and H). At 5C, increasing seed SP had little or no effect on $T_{90}-T_{10}$ spans at an RH $<40\%$ (Fig. 1G); however, at 15C, $T_{90}-T_{10}$ remained low (14 days) over the range of RHs only with <4 months storage (Fig. 1H). At 25C, the lowest $T_{90}-T_{10}$ (≤ 4 days) occurred after storage for <4 months for all RHs. Also, at 25C, a curvilinear effect on $T_{90}-T_{10}$ was found over the range of storage periods. This result indicated that long and short SPs had lower $T_{90}-T_{10}$ than intermediate SPs (Fig. 1I).

Fig. 1. Contour plots for total germination percentages (A-C), days to 50% of final germination (D-F), and days between 10% and 90% germination (G-I) for 'Light Salmon' phlox seeds germinated at 20C following storage at 5C and 11% to 95% relative humidity for 3 to 12 months (A, D, G), 15C (B, E, H), or 25C (C, F, I). Regression equations used to generate these plots are listed in Table 4.

Table 4. Coefficient estimates and significance levels $\Pr(|T| > t_a) = \alpha$ for the fitted regression models. Estimated response = $\beta_0 + \beta_1 SP + \beta_2 RH + \beta_3 SP \times RH + \beta_4 SP^2 + \beta_5 RH^2$.

Response	Temp (°C)	Coefficient estimates						R^2	Figure
		b_0	b_1	b_2	b_3	b_4	b_5		
G	5	90.83	0.45	0.33	-0.02		-0.004	0.6369	1A
	15	70.79	0.0685	0.0002	0.0002		0.0001		
	25	90.26	0.0044	0.0001	0.0001	-0.18	-0.01	0.8698	1B
T_{50}	5	4.22	0.4385	0.0247	0.0109	0.0362	0.0001	0.6514	1C
	15	4.26	-0.05	-0.06	0.004		0.0248	0.8411	1D
	25	4.36	0.0551	0.0001	0.0001		6.3×10^{-4}	0.8912	1E
$T_{90} - T_{10}$	5	1.82	-0.02	-0.06	0.004		6.2×10^{-4}	0.9071	1F
	15	0.74	0.3438	0.0001	0.0001		1.4×10^{-3}		
	25	-1.05	0.02	-0.10	0.008		9.7×10^{-4}	0.7802	1G
	5	0.38	0.6118	0.0001	0.0001	-2.4×10^{-2}	0.0001	0.9048	1H
	15	0.61	0.0031	0.0001	0.0031	0.0021	0.0001		
	25	-1.05	0.0001	0.0025	0.0001	0.0001	5.0×10^{-4}	0.8662	1I
			0.0001	0.6420	0.0014	0.0001			

Having seed that has high total G and rapid and uniform seedling emergence is important to commercial bedding plant producers. Hartmann et al. (1990) reported that alternating daily temperatures 10C improved seed germination in species requiring both high and low temperatures. Our results showed that alternating daily temperatures did not improve phlox germination. Similar total Gs were found for 'Light Salmon' ($G \geq 93\%$) seeds germinated at similar constant or alternating temperatures, while 'Deep Salmon' seeds germinated better ($G \geq 83\%$) at constant temperatures. The T_{50} values for 'Light Salmon' at constant 20 or 25C were shorter than at alternating temperatures, but generally no differences were found among the T_{50} values for 'Deep Salmon'. $T_{90} - T_{10}$ for both cultivars was similar at constant and alternating germination temperatures, ranging from 2.2 to 5.3 for 'Light Salmon' and 4.1 to 6.0 for 'Deep Salmon'. No phlox seed germinated at constant 30C, while alternating 20/30C or 25/30C reduced G to 58% and 36% for 'Light Salmon' and 'Deep Salmon', respectively, and delayed T_{50} and $T_{90} - T_{10}$ (data not presented). Our results explain the low total and irregular germination that occurs when direct-seeded phlox are germinated in warm greenhouses.

In seed production, freshly harvested seeds are dried to low moisture content before they are stored and shipped in sealed packages. It is important to know if the seed moisture content used by seed producers can delay germination or reduce viability or vigor. Our study showed that G was similar when phlox seed moisture

content was between 9% and 5%. Similarly, T_{50} or $T_{90} - T_{10}$ was unaffected at 9% to 6% seed moisture content. At 5% moisture content, however, T_{50} and $T_{90} - T_{10}$ were higher than the corresponding values at higher moisture levels. Bewley and Black (1982) associated cell membrane deterioration with delayed and irregular germination of seeds after they were stored at low moisture content. Justice and Bass (1978) reported that delayed and irregular germination are early indicators of embryo injury. In our research, no embryo injury was observed in histochemically prepared tissue.

'Light Salmon' seed stored for 12 months at 5C and 11%, 34%, 52%, 75%, or 95% RH contained 6.5%, 7.8%, 9.7%, 11.2%, and 13.6% moisture, respectively. The regression analyses estimated that low to intermediate RHs (10% to 50%) and SPs (≤ 6 months) produced the highest total germination percentages and shortest T_{50} and $T_{90} - T_{10}$ at the three temperatures. The smallest amount of change in these germination responses occurred at low RHs (10% to 40%), even when the seeds were stored for 12 months. Our results indicated that the slowest deterioration of phlox seed during storage occurred at a seed moisture content $< 6\%$, the low end of the seed desiccation tolerance range. The highest G and shortest T_{50} and $T_{90} - T_{10}$ generally resulted after seeds were stored at 5C.

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